Title: PREPARATION OF RADIOPHARMACEUTICALS BASED ON TECHNETIUM-99m/

Abstract

A method of reducing a radioactive material comprising, depositing a reducing agent onto an internal surface of a reaction vessel, and contacting a solution of the radioactive material with the surface, to thereby provide a reduced radioactive solution substantially free of reducing agent. Technetium (99mTc) in the form of a solution of pertechnetate ions is reduced by stannous chloride coated on the inside of a reaction vessel, to provide a technetium solution free of excess stannous chloride. A kit for preparing technetium-labelled ligands for use as radio-pharmaceutical is also disclosed.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| AT  | Austria   | GA | Gabon |
| AU | Australia | GB | United Kingdom |
| BB | Barbados | HU | Hungary |
| BE | Belgium | IT | Italy |
| BG | Bulgaria | JP | Japan |
| BR | Brazil | KP | Democratic People's Republic of Korea |
| CF | Central African Republic | KR | Republic of Korea |
| CG | Congo | LI | Liechtenstein |
| CH | Switzerland | LK | Sri Lanka |
| CM | Cameroon | LU | Luxembourg |
| DE | Germany, Federal Republic of | MC | Monaco |
| DK | Denmark | MG | Madagascar |
| FI | Finland | ML | Mali |
| FR | France | MR | Mauritania |
| MW | Malawi | NL | Netherlands |
| NO | Norway | RO | Romania |
| SD | Sudan | SE | Sweden |
| SN | Senegal | SU | Soviet Union |
| TD | Chad | TG | Togo |
| US | United States of America |
This invention relates in general to nuclear medicine, and in particular it relates to radiopharmaceuticals, and to a novel method for their production. Even more particularly, this invention relates to a novel process of producing radiopharmaceuticals comprising suitable ligands "labelled" or "tagged" with radioactive technetium (99m Tc).

Nuclear medicine is a specialised field of medicine practised predominantly in large hospitals which have considerable resources and equipment. Usually, patients present themselves at the hospital with a problem of a diagnostic nature and receive injections of a low dosage radioactive chemical which is known as a radiopharmaceutical. The radiopharmaceutical consists of a radioactive portion which emits radioactivity that can be detected by a suitable detector, and a ligand portion which is non-radioactive and which allows the compound to enter into chemical reactions within the patient's body. The ligand portion is selected from a range of suitable ligands, the choice being dependent upon the
presumed diagnosis of the disease being investigated since it is the chemical properties of the ligand which determine the chemical reactions the radiopharmaceutical will participate in, and hence where in the body it will be distributed. After the injection into a patient of the radioactive chemical, the emitted radiation may be detected by the detector in order to follow the metabolic or other distribution pathways of the administered radioactive chemical. Thus the distribution of the radioactive chemical within the patient's body and throughout the various organs by pathological and physiological processes may be monitored and studied.

The process of attaching the radioactive portion to the ligand portion of the radiopharmaceutical is known as "labelling", and in effect results in the ligand being "tagged". One commonly used radioactive element is technetium 99m (99mTc) (usually available in the form of the pertechnetate ion (TcO₄⁻)) which is metastable and has a half life of around 6 hours, decaying by means of gamma emissions which can be detected by a gamma camera. Thus, when a patient is injected with 99mTc attached to a selected ligand, the distribution of the ligand within the patient's body may be monitored by obtaining an image from the gamma camera of the distribution of the 99mTc in the patient. It is to be noted that throughout this specification, the terms technetium, pertechnetate ion and 99mTc are used to
denote the radioactive portion of the radiopharmaceutical or its precursor.

Technetium (\(^{99m}\text{Tc}\)) is usually obtained from a molybdenum 99 generator in the form of a saline solution of pertechnetate ion, \(\text{TcO}_4^-\), in which the technetium is in the +7 oxidation state. Technetium in this form is not suitable for reaction with the various ligands usually used to form radiopharmaceuticals, and in general it must be reduced to the technetium cation in either the +3 or +4 oxidation state prior to the reaction with the selected ligand. The reduction of technetium is usually achieved by reacting the pertechnetate ion with a stannous chloride (SnCl\(_2\)) solution. It has been found, however, that the stannous chloride solution must be freshly prepared since it readily undergoes atmospheric oxidation, particularly in the presence of saline.

Most \(^{99m}\text{Tc}\) compounds used for in-vivo studies obtained by reduction using stannous chloride are prepared from commercially available kits which involve the direct reaction between the stannous ions, the technetium in the form of the pertechnetate ion, and the selected ligand, with the stannous ions being in excess to ensure that all of the pertechnetate ion is converted to a lower oxidation state. A typical commercially available kit comprises a sealed, sterile container containing a powdered mixture of stannous chloride and the selected ligand. Immediately prior
to use, the seal on a sterile container is broken and a saline solution of pertechnetate ion is added to the container. Reduction of the pertechnetate ions occurs simultaneously with reaction of the reduced technetium cation with the ligand. The container is agitated to ensure complete reaction and a sample of the reaction liquid is withdrawn into a syringe which is then injected into the patient. As the injection solution is taken straight from the container, any unreacted ligand and unreacted excess stannous chloride is injected into the patient along with the desired radiopharmaceutical. Furthermore, excess stannous ions may react with the ligand itself rather than with the pertechnetate ions and therefore prevent, or hinder, the desired reaction between the reduced technetium cation and the ligand. The free stannous chloride injected into the patient as discussed above can enter into unwanted metabolic reactions and alterations during and after the studies on the patient such as, for example, following a bone scan using technetium-labelled methylendiphosphonate. Furthermore, stannous chloride present in the general body circulation makes it difficult to perform some other scan studies on the same patient within a reasonably short time.

Therefore, it is an aim of the present invention to at least alleviate some of the problems associated with the prior art methods of making radiopharmaceuticals, particularly those which consist of radioactive technetium-labelled ligands.
United States Patent Specification No. 4,272,503, issued June 9, 1981, discloses a reductant composition for technetium 99m and a method for making technetium 99m labelled ligands. In particular, this specification discloses in one embodiment a reductant for reducing technetium comprising a substrate having a reducing complex attached thereto, the reducing complex preferably comprising a reducing agent and a chelating ligand for binding the reducing agent to the substrate. The chelating ligand may be any known chelating ligand for the reducing agent and may be bound to the substrate either directly or through a linking group. Alternatively, substrates having chelating ligand already bound thereto, for example, those commercially available as functionalized glass beads or functionalized polysaccharide beads, may be used. It is an object of the present invention to provide an alternative method of making radioactive-labelled ligands which is simpler and more economical than the method described in the above mentioned patent specification and yet which provides an effective means for producing the desired radiopharmaceuticals.

In general terms, the present invention provides a method of overcoming the unwanted effects of excess stannous chloride by depositing stannous chloride on the surface of a reaction vessel, for example, as a thin layer, coating or the like, and then reducing the pertechnetate ion in this reaction vessel so that there is no excess stannous chloride in
the solution injected into the patient's body.
Further advantages of this method are that unlike the
established methods, ligands which are unstable in
dilute acids or which precipitate with tin ions or
which are insoluble in saline can still be combined
with technetium to form radiopharmaceuticals.

According to one aspect of the present
invention there is provided a method of reducing a
radioactive material, particularly reducing
pertechnetate ion to Tc(III) or Tc(IV), which method
comprises the steps of depositing a suitable reducing
agent onto a surface, for example the internal wall of
a reaction vessel, and reacting the radioactive
material with the reducing agent by contacting a
solution containing the radioactive material with the
surface thereby reducing the radioactive material and
providing a solution containing reduced radioactive
material substantially free of reducing agent.

In contrast to the method disclosed in U.S.
Patent Specification No. 4,272,503, in the present
method the reducing agent is deposited directly onto
the substrate surface, and accordingly the need to
prepare a "reducing complex" between the reducing
agent and a chelating ligand therefor, or to use a
special functionalized substrate, is avoided.

Preferably the reducing agent is a source of
stannous ions, particularly stannous chloride. Other
known reducing agents may, however, be used, including
for example, ferrous ions, cuprous ions, ferric-ascorbate complexes, and reduced zirconium. Furthermore, salts other than the chloride salts, such as pyrophosphate salts, may also be used. The surface onto which the reducing agent is deposited may be either a glass surface or a plastics surface such as polyethylene. In a particularly preferred embodiment the surface is the inner wall of a reaction vessel, such as a syringe or a length of tubing.

According to another aspect of the present invention there is provided a method of preparing a radioactive labelled ligand substantially free of reducing agent, particularly a technetium-labelled ligand substantially free of reducing agent, comprising the steps of:

(a) depositing a reducing agent onto the internal surface of the vessel;

(b) admitting a radioactive material to the vessel;

(c) admitting a ligand to be labelled by the radioactive material to the vessel;

(d) reacting the radioactive material, ligand to be labelled and reducing agent to reduce the radioactive material and form a radioactive labelled ligand whilst substantially retaining the reducing agent on the internal surface of the reaction vessel.

If desired, a silicon-containing material such as dimethyl-siloxane may be applied to the internal surface of the reaction vessel before
deposition of the reducing agent, however this is not essential.

According to another aspect of the present invention there is provided a kit for producing a radioactive labelled ligand suitable for use as an injectable radiopharmaceutical comprising a reaction vessel having a reducing agent deposited on an inner surface thereof, means for admitting a radioactive material to the reaction vessel and means for admitting a ligand to be labelled by the radioactive material to the reaction vessel, whereby the radioactive material is reduced and ligand is labelled by the reduced radioactive material within the reaction vessel, and means for discharging the radioactive-labelled ligand substantially free of the reducing agent from the reaction vessel.

According to another aspect of the present invention there is provided a radioactive labelled ligand, particularly a radioactive technetium-labelled ligand, whenever prepared by a method of the present invention.

Any ligand capable of being labelled, for example with technetium 99m, can be labelled in accordance with this invention. Particularly useful ligands are polyhydroxy polycarboxylic acids, aminocarboxylic acids, phosphonates, phosphates and mercaptans, etc. Examples of such ligands include, for instance, plasma proteins such as human serum
albumin (HSA), ethylhydroxydiphosphonate (EHDP),
methylenediphosphonate (MDP), pyrophosphate,
ethylenediaminetetraacetic acid (EDTA),
diethylenetriaminepentaacetic acid (DTPA),
dimercaptosuccinic acid (DSMA), gluconate,
glucoheptonate, N-(2,6-dimethylphenylcarbamoylmethyl)
iminodiacetic acid (HIDA), analogs of HIDA such as
N-(2,6-diisopropylphenylcarbamoylmethyl)iminodiacetic
acid (PRIDA), N-(4-butylphenylcarbamoylmethyl)-
iminodiacetic acid (BIDA), clotting factors such as
fibrinogen, gamma globulins, antibodies and their
fractions, phytate, and the like.

The present invention will now be described
by way of example with reference to the accompanying
drawings in which:

FIGURE 1 is a bone scan of rabbit using
$^{99m}$Tc-labelled methylene diphosphonate (MDP) prepared
in accordance with the present invention shown in
three different intensities;

FIGURE 2 is a bone scan similar to Figure 1 using
$^{99m}$Tc-labelled methylene diphosphonate prepared by
commercially available kits also shown in three
different intensities;

FIGURE 3 is a kidney scan of rabbit using
$^{99m}$Tc-labelled diethylenetriamine penta-acetic acid
(DTPA) prepared in accordance with the present
invention shown in three different intensities.

Technetium in the form of pertechnetate ion
($^{99m}$TcO$_4^-$) in a saline solution is obtained by elution
from a sterile $^{99m}$Mo-$^{99m}$Tc generator, such as for
example, a Mallinckrodt Aiishi generator. A solution having typically 50 to 100 mCi $^{99m}$TcO$_4^-$ in saline solution is used in the subsequent labelling reaction.

**Preparation of Vessel with thin coating of stannous chloride**

Any suitable material such as glass or plastic may form a suitable reaction vessel. However, in the described embodiment a sterile non-pyrogenic polyethylene catheter pipette was used, the internal surface of the pipette was washed with dimethyl-siloxane in order to make the internal bore of the catheter smooth by depositing silicon on it. (In alternative embodiments, a polyethylene syringe may be used as the reaction vessel in place of the catheter pipette.) Typically, a solution of Sigmacote which is a commercially available silicone solution from Sigma Inc. may be used. It should be noted that siliconizing the surface of the reaction vessel is an optional step, although siliconised vessels have been found to assist in some instances in prevention of microcolloid formation. The vessel is then dried at room temperature and afterwards rinsed with a normal saline solution. The vessel, whether pretreated with silicon or not, is coated with stannous chloride by using a plastic syringe to flush the stannous chloride solution which has been previously freshly prepared backwards and forwards through the vessel before returning the solution back into the vial containing the solution. Thus, stannous ions are directly deposited on the inside of the vessel by means of a
combination of physical and chemical mechanisms. It is thought that stannous and chloride ions are held inside the vessel by the combined effect of the ions lodging in minute surface troughs in the internal bore of the vessel and the chemical attraction between the ions and the surface. As previously mentioned, it has been discovered that a siliconised plastic surface or smooth glass surface in some instances inhibits and may even eliminate, microcolloid formation which is caused by unlabelled free TcO$_4^-$ and reduced uncomplexed technetium.

In order to prevent residual excess free stannous chloride from remaining inside the vessel the vessel is rinsed with typically 5 ml of normal saline solution (0.9% NaCl). After thus depositing the stannous chloride in the vessel, the vessel is quickly dried at room temperature (to stop autooxidation) in preparation for its use in the next step. Typically, it may be dried with a purge of nitrogen or other conventional means.

Formation of Technetium attached to a ligand i.e. labelling

The process of attaching the radioactive portion to the ligand is known as labelling. A mixture is prepared by mixing an aqueous solution of the ligand and the previously eluted pertechnetate ion saline solution. No reaction between these two reactants takes place since the technetium is in the +7 oxidation state and accordingly it will not readily react with the ligand. The mixture is then passed
through the previously prepared vessel by means of a plastic syringe which is used to flush the mixed solution backwards and forwards repeatedly through the vessel. The simultaneous reduction of Tc and combination of the ligand and Tc takes place in the presence of the SnCl₂ on the walls of the vessel. The SnCl₂ stays substantially on the vessel walls at all times and does not enter the saline solution of Tc and ligand being flushed through the vessel. Therefore, no SnCl₂ can enter into the solution to be injected into the patient.

By this means the pertechnetate ion is reduced and the ligand complex is formed i.e. the ligand is labelled by the technetium.

Verification of the Invention

The analytic method employed to verify that a solution substantially free of SnCl₂ is obtained is thin-layer chromatography using saline and methyl ethyl ketone as solvents.

After the reduction of the pertechnetate ion by the stannous chloride there exist in solution the following species (i) free unreacted pertechnetate ion TcO₄⁻, (ii) reduced but uncomplexed technetium in the form of ⁹⁹ᵐTc(III) or ⁹⁹ᵐTc(IV), and (iii) technetium labelled ligand. Saline solution is used to separate the reduced uncomplexed Tc from the unreacted pertechnetate ion and the labelled ligand. Methyl ethyl ketone is used to separate the free unreduced pertechnetate ion from the technetium
labelled ligand and the reduced uncomplexed technetium.

Generally, the stationary phase used in the chromatographic process is prepared from pure cellulose sheets of chromatographic quality such as Watman No. 1 which are cut into strips typically about 7 mm wide by 57 mm long. These strips which are used with the saline medium are marked transversely with a graphite pencil about 10 mm from one end. Similarly, the strips which are used with the methyl ethyl ketone are marked about 10 mm from the same one end.

For each labelling two strips are used. One drop of the labelled compound under test is placed on strip A and one drop of the same compound is placed on strip B. Without drying, strip A is inserted vertically into a flat-bottomed vial containing saline solution to a depth of about 2 to 3 mm and strip B is inserted into a flat bottom vial containing methyl ethyl ketone to a depth of about 2 to 3 mm. Just before the solvent front reaches the top of each chromatographic strip (2-3 min), the strips are removed from the vial. They are then cut at the pencil marks and the two components are placed in counting tubes for measurement in a well counter or a dose calibrator. These sections are designated as $A_1$ and $A_2$ for strip A and $B_1$ and $B_2$ for strip B.

The percentages of reduced uncomplexed $^{99m}$Tc, free pertechnetate ion and $^{99m}$Tc labelled ligand are calculated from the counts obtained, using the following formulas:
Reduced uncomplexed $^{99m}$Tc = \( \frac{A1}{A1+A2} \times 100 = R \)

Free pertechnetate ion = \( \frac{B2}{B1+B2} \times 100 \% = F \)

$^{99m}$Tc Labelled = 100 - (R + F)

All of components are tested and measured by this method (Table 1).
<table>
<thead>
<tr>
<th>COMPOUNDS (Tc)</th>
<th>Concentration (mg/ml)</th>
<th>pH</th>
<th>Free Per tech used</th>
<th>Percentage 99mTc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Citrate</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>2. Pyrophosphate</td>
<td>5</td>
<td>7</td>
<td>0.0</td>
<td>1.76</td>
</tr>
<tr>
<td>3. EDTA (Ethylenediamine Tetra-acetic Acid)</td>
<td>10</td>
<td>6.5-7</td>
<td>0.0</td>
<td>1.9</td>
</tr>
<tr>
<td>4. HIDA</td>
<td>5</td>
<td>6.5</td>
<td>0.9</td>
<td>2.1</td>
</tr>
<tr>
<td>5. DTPA (Diethylentriamine Penta-acetic Acid)</td>
<td>5</td>
<td>6</td>
<td>0.3</td>
<td>2.2</td>
</tr>
<tr>
<td>6. MDP (Methylenediphosphonate)</td>
<td>2</td>
<td>5-7</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>7. Bleomycin</td>
<td>$2 \times 10^{-3}$</td>
<td>7</td>
<td>12.5</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>$4 \times 10^{-3}$</td>
<td>7</td>
<td>7.7</td>
<td>6.3</td>
</tr>
<tr>
<td>8. BSA (Bovine Serum Albumin)</td>
<td>1</td>
<td>7</td>
<td>23.5</td>
<td>25.0</td>
</tr>
<tr>
<td>9. Diethyl-Dithio-carbonate (non polar)</td>
<td>5</td>
<td>6-7</td>
<td>22.5</td>
<td>23.1</td>
</tr>
<tr>
<td>10. Ethylendibis dithio-carbonate</td>
<td>5</td>
<td>6-7</td>
<td>25</td>
<td>18.7</td>
</tr>
<tr>
<td>11. Glucosamine-1,2.bis (thiosemicarbazon)</td>
<td>3</td>
<td>5-6</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>12. Oxalate</td>
<td>3</td>
<td>6.5</td>
<td>16.3</td>
<td>8</td>
</tr>
<tr>
<td>13. Gluconate</td>
<td>3</td>
<td>6-7</td>
<td>2.4</td>
<td>27.5</td>
</tr>
<tr>
<td>14. Ammonium chloride</td>
<td>1</td>
<td>6-7</td>
<td>2.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

(Siliconized catheter not used in 1, 4, 9, 10, 12 and 13)
As illustrated in Table 1 the technique of the present invention employed 14 different chemical compounds which are usable in nuclear medicine for diagnosis. Some of the ligands used in the method of the present invention include citrate which is used in the study of tumours; pyrophosphate which is used in the study of bones; ethylenediaminetetraacetic acid which is used in the study of kidneys; diethylenetriamine penta-acetic acid which is used in the study of kidneys also generally ligands labelled with technetium are useful in the diagnosis of cancer, particularly metastas and in the dynamic study of kidneys, particularly kidney failure.

As mentioned below, the effects of pH and Stannous chloride concentration which are two important aspects of the reaction of the present invention were investigated.

**Effect of pH**

Solutions containing technetium and the various ligands were made at various pHs and checked more than five times. (Table 1 shows the mean value of pH for each compound), then mixed with 0.5 - 1mCi/ml (18.2 - 37.0 MBq) of TcO₄⁻. The ligands are usually soluble in normal saline and stirred for a few minutes. pH is adjusted by using alkaline solution, 0.1 M NaOH or acid solution, HCl 1 M. The solution of the ligand to be labelled is filtered through a 0.22 μm Millipore filter. The highest efficiency for complex formation
is obtained at pH 5 - 7. In this procedure the formation of free unreacted $^{99m}$TcO$_4^-$ and uncomplexed $^{99m}$TcO$_2$ was very low for certain compounds and negligible for most of them. Reference may be made to Table I in this regard where the percentages of each of (i) free pertechnetate ion, (ii) reduced uncomplexed technetium, and (iii) labelled technetium are given in the appropriately headed columns at the right hand side of the table.

**Effect of the concentration of Stannous chloride**

Various concentrations of aqueous stannous chloride solutions are made and filtered through 0.22 \mu m Millipore filter material. After a few experiments, the concentration of 1mM is used for all compounds apart from red blood and platelet studies where a concentration of less than 1 was used.

**Use of other reducing salts**

As previously described, reducing salts other than stannous chloride can be used for preparation of certain labelled ligands. By way of example, Tc-red blood cell labels prepared by the method of the present invention using stannous chloride as the reducing agent were found to be unstable in vivo. However, where stannous pyrophosphate (10\mu g/ml) was deposited as the reducing agent on the internal surface of a syringe then
suitably labelled red blood cells were obtained in good yield (76%) with excellent in vivo stability. Other stannous salts including Sn-oxime and Sn-EDTA may also be used.

Stability of deposited reducing agent

Polyethylene syringes having Sn·Cl₂ deposited on the internal surface thereof in accordance with this invention were stored in air at -10°C for periods of three and six months. Following this storage period, these syringes were used in the preparation of labelled ligands by the method of this invention, using sodium pertechnate and DTPA, MDP and PYP as ligands. Labelling yields (calculated as previously described) are set out in Table 2.
### TABLE 2

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Syringe stored for 3 months - labelling yield (%)</th>
<th>Syringe stored for 6 months - labelling yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPA</td>
<td>96.6 ± 1.8</td>
<td>92.0 ± 1.3</td>
</tr>
<tr>
<td>MDP</td>
<td>95.0 ± 1.3</td>
<td>90.6 ± 2.5</td>
</tr>
<tr>
<td>PYP</td>
<td>96.2 ± 2.1</td>
<td>92.0 ± 2.6</td>
</tr>
</tbody>
</table>
Study of Rabbits

Solutions obtained by the method of the present invention were injected into rabbits which were used for in vivo studies. The results of the scans are shown in Figures 1 to 3. The Gamma camera used for obtaining the images of the scans was a Toshiba GLA402. For example, the radiopharmaceutical Tc-labelled DTPA (Figure 3) was intravenously injected into the rabbit's ear in an amount of 250 μCi (9 MBq). Following the intravenous injection into the rabbit, $^{99m}$Tc-DTPA is rapidly cleared from the blood circulation by excretion into urine. Kidney uptake was clear as shown in Figure 3. There is no accumulation or retention in the thyroid gland or salivary glands as illustrated. The bladder only was visualised in the scan.

As a further example, and as a comparison, a commercially prepared as well as a surface labelled Tc MDP complexes were injected intravenously into two rabbits. A bone scan was performed two hours after the injection. Figures 1 and 2 show the relative quality of each of the scans.

The present invention provides a method producing a simpler way of obtaining technetium-labelled compounds, and of obtaining many more technetium compounds than has hitherto previously been obtainable by conventional commercial methods.
This method is of commercial value in nuclear medicine where on a world-wide basis millions of dollars worth of compounds which are used for labelling with technetium are sold annually.

One of the advantages of the present invention is that there is none or at worst a negligible amount (about < 4 μg) of stannous chloride injected into the biological system of the patient receiving the invention. Accordingly, there is less chance of there being adverse side reactions in the patient and interference with subsequent treatments and investigations.

The method of the present invention provides a simple and reliable method which offers considerable promise in combining various compounds (ligands) with technetium using the stannous ions that hitherto have not been possible or entirely successful due to the ligands precipitating out of solution or due to pH incompatibility. Therefore, although the present invention has been described for some ligands with technetium, many other ligand reactions are possible and it is applicable to other radioactive materials other than technetium.

These and other modifications may be made without departing from the spirit and scope of the invention which includes every novel feature and combination of novel features herein disclosed.
CLAIMS

1. A method of reducing a radioactive material, which method comprises the steps of depositing a suitable reducing agent onto a surface, and reacting the radioactive material with the reducing agent by contacting a solution containing the radioactive material with the surface thereby reducing the radioactive material and providing a solution containing reduced radioactive material substantially free of reducing agent.

2. A method according to claim 1, wherein the surface onto which the reducing agent is deposited is the internal wall of a reaction vessel.

3. A method according to claim 1, wherein the radioactive material comprises pertechnetate ions, which are reduced to Tc(III) or Tc(IV).

4. A method according to claim 1, wherein the reducing agent comprises stannous ions.

5. A method of preparing a radioactive labelled ligand substantially free of reducing agent, comprising the steps of:
   (a) depositing a reducing agent onto the internal surface of the vessel;
   (b) admitting a radioactive material to the vessel;
(c) admitting a ligand to be labelled by the radioactive material to the vessel;
(d) reacting the radioactive material, ligand to be labelled and reducing agent to reduce the radioactive material and form a radioactive labelled ligand whilst substantially retaining the reducing agent on the internal surface of the reaction vessel.

6. A method according to claim 5, wherein the radioactive material comprises pertechnetate ions, which are reduced to Tc(III) or Tc(IV).

7. A method according to claim 5, wherein the reducing agent comprises stannous ions.

8. A method according to claim 5, wherein the vessel comprises a glass or plastics syringe or length of tubing.

9. A kit for producing a radioactive labelled ligand suitable for use as an injectable radiopharmaceutical comprising a reaction vessel having a reducing agent deposited on an inner surface thereof, means for admitting a radioactive material to the reaction vessel and means for admitting a ligand to be labelled by the radioactive material to the reaction vessel, whereby the radioactive material is reduced and ligand is labelled by the reduced radioactive material within the reaction vessel, and means for discharging the radioactive-labelled ligand.
24

...substantially free of the reducing agent from the reaction vessel.

10. A radioactive labelled ligand, prepared by the method according to claims 5 to 8, or by use of the kit according to claim 9.
1. (Amended) A method of preparing a radioactive labelled ligand, which method comprises the steps of depositing a suitable reducing agent onto a surface, and reacting a solution containing a radioactive material with the reducing agent in the presence of the ligand to be labelled by contacting said radioactive material with the surface in the presence of said ligand, thereby reducing the radioactive material and providing said ligand labelled with reduced radioactive material substantially free of reducing agent.

2. A method according to claim 1, wherein the surface onto which the reducing agent is deposited is the internal wall of a reaction vessel.

3. A method according to claim 1, wherein the radioactive material comprises pertechnetate ions, which are reduced to Tc(III) or Tc(IV).

4. A method according to claim 1, wherein the reducing agent comprises stannous ions.

5. (Amended) A method of preparing a radioactive labelled ligand substantially free of reducing agent, comprising the steps of:
   (a) depositing a reducing agent onto the internal surface of the vessel;
(b) admitting a ligand to be labelled by a radioactive material to the vessel;
(c) simultaneously with or subsequent to step (b), admitting said radioactive material to the vessel;
(d) reacting the radioactive material, ligand to be labelled and reducing agent to reduce the radioactive material and form a radioactive labelled ligand whilst substantially retaining the reducing agent on the internal surface of the reaction vessel.

6. A method according to claim 5, wherein the radioactive material comprises pertechnetate ions, which are reduced to Tc(III) or Tc(IV).

7. A method according to claim 5, wherein the reducing agent comprises stannous ions.

8. A method according to claim 5, wherein the vessel comprises a glass or plastics syringe or length of tubing.

9. (Amended) A kit for producing a radioactive labelled ligand suitable for use as an injectable radiopharmaceutical comprising a reaction vessel having a reducing agent deposited on an inner surface thereof, means for admitting a radioactive material and a ligand to be labelled by the radioactive material to the reaction vessel, whereby the radioactive material is reduced and said ligand is
labelled by the reduced radioactive material within the reaction vessel, and means for discharging the radioactive-labelled ligand substantially free of the reducing agent from the reaction vessel.

10. A radioactive labelled ligand, prepared by the method according to claims 5 to 8, or by use of the kit according to claim 9.
FIGURE 3
INTERNATIONAL SEARCH REPORT  
International Application No PCT/AU 84/00253

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) 3
According to International Patent Classification (IPC) or both National Classification and IPC
Int. Cl. 3 C01G 57/00, A61K 49/02

II. FIELDS SEARCHED

<table>
<thead>
<tr>
<th>Classification System</th>
<th>Minimum Documentation Searched 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPC</td>
<td>C01G 57/00, A61K 49/02, 43/00</td>
</tr>
<tr>
<td>US Cl.</td>
<td>424/1, 424/1.1</td>
</tr>
</tbody>
</table>

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 5
AU : IPC as above & Q01N 33/60

III. DOCUMENTS CONSIDERED TO BE RELEVANT 14

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, 14 with Indication, where appropriate, of the relevant passages 17</th>
<th>Relevant to Claim No. 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>AU, B, 39680/78 (SOLCO BASEL A.G.) 13 August 1981 (13.08.81) See claims 5 &amp; 7 &amp; page 7.</td>
<td>(1,9)</td>
</tr>
<tr>
<td>X</td>
<td>US, A, 4314986 (RUDDOCK) 9 February 1982 (09.02.82) See page 4 lines 30 - 32 and claim 8.</td>
<td>(1,9)</td>
</tr>
<tr>
<td>X</td>
<td>US, A, 4311698 (RUDDOCK) 19 January 1982 (19.01.82) See claims 6 &amp; 9 and page 4 lines 35 - 37.</td>
<td>(1,5,9)</td>
</tr>
<tr>
<td>X</td>
<td>US, A, 4272503 (GAMIN ET AL) 9 June 1981 (09.06.81) See claims 6 &amp; 31.</td>
<td>(1,5,9)</td>
</tr>
<tr>
<td>A</td>
<td>US, A, 4401646 (RHODES ET AL) 30 August 1983 (30.08.83)</td>
<td></td>
</tr>
</tbody>
</table>

* Special categories of cited documents: 15
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "E" earlier document but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search 9 18 February 1985 (18.02.85)
Date of Mailing of this International Search Report 1 7 March 1985 (07.03.85)
International Searching Authority 1 AUSTRALIAN PATENT OFFICE
Signature of Authorized Officer 10 R.E. GRANT

Form PCT/ISA/210 (second sheet) (October 1981)
ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 84/00253

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cited in Search Report</td>
<td></td>
</tr>
<tr>
<td>AU 517648</td>
<td>BE 870219 CA 1108049 CH 630261</td>
</tr>
<tr>
<td></td>
<td>DE 2838753 UK 3950/78 ES 473146</td>
</tr>
<tr>
<td></td>
<td>FI 782746 FR 2402447 GB 2006510</td>
</tr>
<tr>
<td></td>
<td>JP 54052298 NL 7809172 NO 782982</td>
</tr>
<tr>
<td></td>
<td>SE 7809327 US 4250161</td>
</tr>
<tr>
<td>US 4314986</td>
<td>DE 3007402 FR 2450234 GB 2046000</td>
</tr>
<tr>
<td></td>
<td>JP 55116630</td>
</tr>
<tr>
<td>US 4311689</td>
<td>DE 2907880 FR 2418648 GB 2016198</td>
</tr>
<tr>
<td></td>
<td>JP 54126597</td>
</tr>
<tr>
<td>US 4272503</td>
<td>BE 876562 CA 1111765 DE 2920770</td>
</tr>
<tr>
<td></td>
<td>FR 2430236 GB 2023327 JP 54160305</td>
</tr>
<tr>
<td></td>
<td>NL 7904059 SE 7904316</td>
</tr>
<tr>
<td>US 4401646</td>
<td>CA 1177392 EP 65347</td>
</tr>
</tbody>
</table>

END OF ANNEX